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NUTRITIONAL PROPERTIES OF THE MOLECULARLY DISTILLED FRACTIONS OF AUTOXIDIZED FATS [1]

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In previous nutritional work on autoxidized fats, special attention was paid either to the polymer fraction left as a residue after molecular distillation (Kaunitz et al., '55) or to the whole autoxidized fat. It was found that the effects of the polymer fraction or of the whole autoxidized fat could be counteracted by the addition of fresh fat to the diet (Kaunitz et al., '55) and that the caloric requirement of the rat for weight maintenance was increased when such fats were consumed.

The distillate fraction of autoxidized fats, obtained by molecular distillation, had previously been studied only briefly and had not seemed to be particularly remarkable. The further studies to be reported below, however, show that this fraction is also of interest nutritionally.

EXPERIMENTAL

The studies were carried out on albino rats of a homogeneous colony. Weanling males, when they weighed 40 to 50 gm,

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were placed on a purified diet containing 30% of lactalbumin and 10% of fresh lard. At the age of 5 weeks, they were distributed into matching groups using procedures reported before (Kaunitz et al., '54).

Commercial lard and refined cottonseed oil were aerated at 95°C. for 200 to 300 hours and then distilled, using alembic distillation for the removal of volatile products, followed by molecular distillation. For the latter, temperatures up to 280°C. were employed. In one instance, a hydrogenated cottonseed oil which had been used for deep fat frying for 80 hours at 190°C. was distilled.

Unless otherwise stated, the experimental diets contained 30% alcohol-extracted casein, 10% fat, 54% dextrose, 4% salts (U.S.P. no. 2), and 2% cellulose, as well as liberal amounts of all known accessory food factors [4] in amounts described before (Kaunitz et al., '54).

RESULTS AND DISCUSSION

In figure 1 are given the average growth curves of groups of 8 male rats which had been maintained on diets containing various fats. The logarithm of the weight in grams is plotted against the reciprocal value of the age; the advantages of this method have been pointed out by Zucker and Zucker ('42).

The animals receiving autoxidized cottonseed oil lost weight rapidly and died after two to 4 weeks. When 10% of fresh fat was added to the diet containing 10% of the oxidized oil, none of the animals died during the period of observation; they were even able to grow. This has previously been described as the protective effect of fresh fat. One group of animals received 10% of the distillate from the molecular distillation of the sample of hydrogenated cottonseed oil which had been used for deep fat frying. These animals grew essentially as well as did those on fresh cottonseed oil. How-

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ever, when the distillate was combined with oxidized cottonseed oil, growth was significantly below that of the animals receiving both fresh and oxidized cottonseed oils. Also, in contrast to the latter group, some of the animals died toward

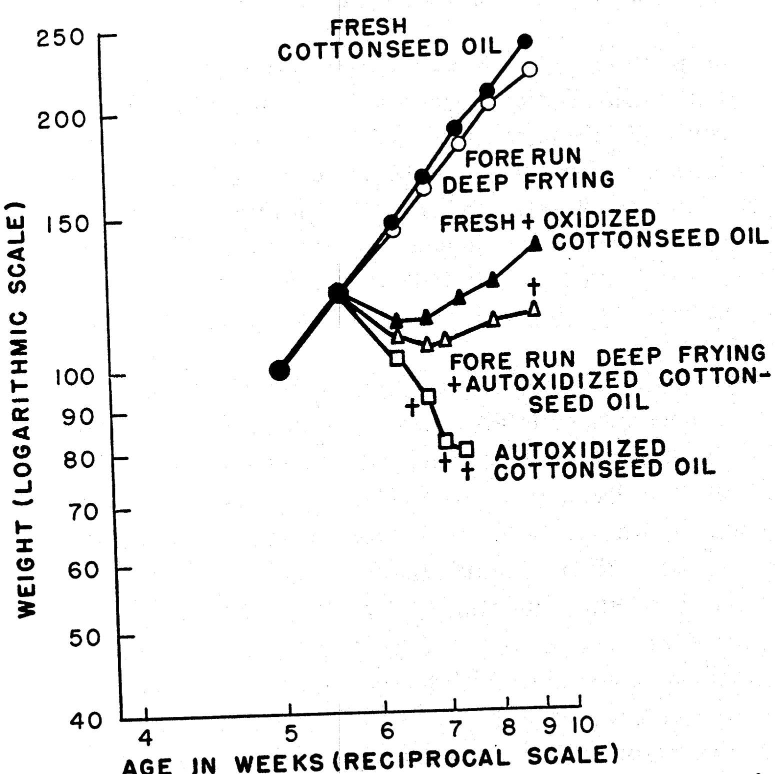

Fig. 1 Influence of the distillate from the molecular distillation of a hydrogenated vegetable oil after its use for deep frying for 80 hours. Each curve is based on the average of 8 well-matched male rats. After the third week of the experiment, the difference in weight of the groups fed oxidized plus fresh fat and oxidized plus distillate is statistically significant.

the end of the period of observation. Therefore, the distillate, while permitting nearly normal growth when included in the diet as the only fat, had lost a high degree of its protective effect.

Six very similar experiments were carried out with the molecular distillation fractions of highly autoxidized cottonseed oil or highly autoxidized lard. These distillates usually permitted good, although not quite optimum, growth when used as the sole fat source. Significantly, all of the distillates had lost their protective effect against highly autoxidized cottonseed oil to a degree very similar to that shown in figure 1.

This loss of protective effect could not have been caused by the molecular distillation process. The undistilled autoxidized cottonseed oil containing 40% polymeric "residue" and 60% "distillate" fraction led to rapid deterioration of the animals, whereas a mixture of 40% polymeric residue and 60% fresh oil permitted acceptable growth. Thus, the lack of protective action of the distillate was discernible before the oil had undergone the heating necessary for molecular distillation.

When rats, by daily weighing and restricted feeding, are maintained at a weight constant within 3 gm, it has been observed that the caloric requirements for such weight maintenance decline rapidly within the first few weeks if "good" diets are used (Quimby, '48). It has been shown (Kaunitz et al., '56) that the caloric requirements for weight maintenance do not decrease when the residue fraction of a molecularly distilled autoxidized fat is included in the diet. In figure 2 is shown a similar experiment with fresh fat and the molecular distillate of the hydrogenated vegetable oil which had been used for deep frying. The requirements are expressed as weekly calories per gram of body weight and are the average values for each group of 8 animals. For the calculation of the caloric values of the diets, it was assumed that a factor of 9.2 Cal. per gram could be used for both fats. It seemed reasonable to assume that the caloric value of the distillate did not differ greatly from that of normal fat because, when the distillate was included in a diet as the only fat source and the animals were permitted to eat freely, (1) the resulting growth was only slightly below that of animals fed fresh fat and (2) the food intakes were similar. However, even if the caloric value of the distillate is slightly below that of the fresh lard, this would not lead to different conclusions.

As can be seen from figure 2, the caloric requirements of both groups declined steeply during the first 4 weeks of observation. The net energy value of the diet containing distillate was lower than that of the diet containing fresh fat, although the difference was not as pronounced as that between the groups fed polymeric residue and fresh fat. However, it

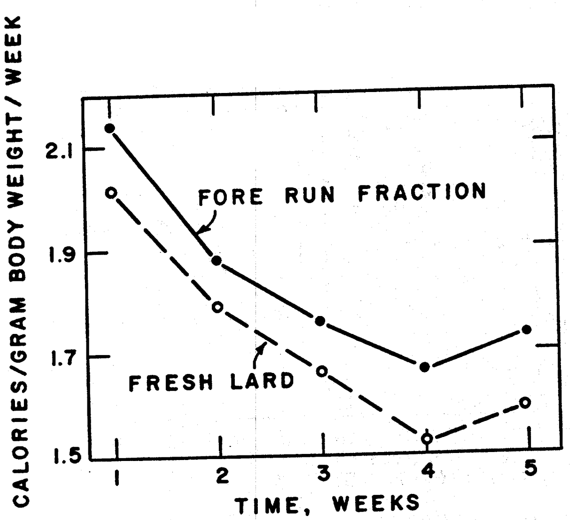

Fig. 2 Influence of fresh lard and the distillate from the molecular distillation of a hydrogenated vegetable oil previously used for deep frying for 80 hours upon the caloric requirements of matching male rats maintained at constant weight. Each curve is based on the average of 8 animals.

may be of some interest that, with a chemically altered but essentially atoxic fat, the animal's caloric requirement for weight maintenance is increased.

When the animals maintained at constant weight were sacrificed at the end of the experiment, their kidneys, livers, and adrenals were weighed. Figure 3 shows log — log plots of organ weights against body weights. The parallel lines give the limits of the spread in organ weight of male rats fed a complete, unrestricted diet. The weights of the livers and kidneys of the animals on the distillate were within normal limits, although somewhat above those of the animals fed fresh fat. The adrenals of the two groups scarcely differed from one another. In contrast, the livers, kidneys, and adrenals of the animals given the residue fraction substantially

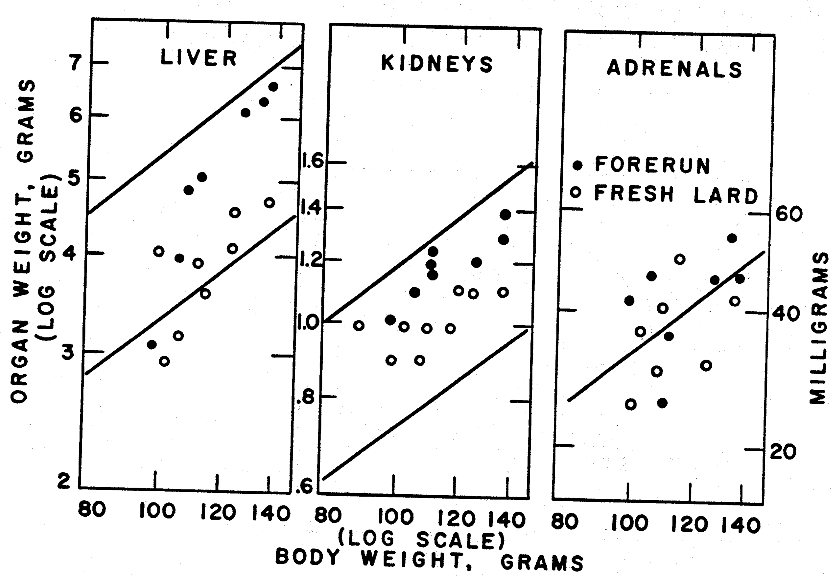

Fig. 3 Influence of fresh lard and distillate from the molecular distillation of a hydrogenated vegetable oil previously used for deep frying for 80 hours upon the organ weight-body weight relationships of male rats kept at constant weight by restricted feeding for 5 weeks. On the log — log plot, the parallel lines indicate the upper and lower limits of the spread in organ weight of rats with unrestricted intakes of a control diet containing fresh lard.

exceeded the upper limit of the normal (Kaunitz et al., '56). These results also show that the distillate itself is hardly toxic.

This low toxicity again became evident in studies with low-protein diets. In earlier work (Kaunitz, '53), it was pointed out that weanling rats placed on diets containing only 5% of casein and fresh fat maintained their weight for several weeks and grew slowly thereafter. Ten per cent of a sample of oxidized lard which was atoxic to rats when included in a diet containing 30% of casein led to rapid weight loss and death when fed in a diet containing only 5% of casein. When 10% of the distillate was included in a diet with 5% of casein, growth of the rats was similar to that of the controls receiving fresh fat.

The chemical changes in the fats responsible for the described effects are not as yet understood. This problem is being actively investigated.

SUMMARY

1. Lard and refined cottonseed oil which had been aerated at 95°C. for 200 to 300 hours and a sample of hydrogenated vegetable oil which had been used commercially for deep fat frying for 80 hours at 190°C. were molecularly distilled at 280°C. The distillates were used in nutritional experiments.

2. When the distillates were included in purified diets containing either 5 or 30% casein, the resulting growth of most of the weanling male rats fed these diets was only slightly below that of matching rats receiving fresh lard.

3. In contrast, distillate added to the nonvolatile polymeric residue from the molecular distillation of autoxidized fats had a protective effect markedly below that of fresh fats.

4. The net energy value of the diet containing distillate was lower than that of the diet containing fresh fat.

5. Liver, kidney and adrenal weights of rats fed distillate were within the normal spread for these organs and were only slightly higher than those of the controls, thereby supplying additional evidence for the low toxicity, if any, of these fractions.

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Doctor Waldo C. Ault of the eastern Regional Laboratory of the U. S. Department of Agriculture has greatly helped this work with his advice, suggestions, and criticisms.

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